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Encapsulation of Probiotic Lactic Acid Bacteria in Pectic Gel Particles

Elena A. Mikhailova[1*], Anatoly A. Shubakov[2]

[1]*Institute of Physiology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS*
[2]*Institute of Biology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS*
Syktyvkar, Komi Republic, the Russian Federation

Abstract

The purpose of this work was to research the encapsulation of lactic acid bacteria (LAB) of the probiotic "Evitalia" in pectic gel particles (PGPs) formed on the basis of apple pectin and citrus pectin.

Methods and Results: Commercial apple pectin (AP) AU701 (Herbstreith & Fox KG, Germany) and citrus pectin (CP) CU701 (Herbstreith & Fox KG, Germany) were used. Gel particles were prepared from 3% aqueous solutions of pectins in the presence of 0.34M $CaCl_2$ by ionotropic gelation. The diameter and density of PGPs were determined using an optical microscope (ALTAMI, Russia). For encapsulation in PGPs, a complex of "Evitalia" dry probiotic microorganisms was used, which consists of freeze-dried strains *of Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus helveticus,* and *Propionibacterium freudenreichii ssp. shermanii*. To accumulate cells of probiotic LAB, we used a modified MRS medium. An extrusion method was used to encapsulate probiotic cells in PGPs. In the study of probiotic-encapsulated PGPs, they were preliminarily destroyed by ultrasound in an HD2070 ultrasonic homogenizer (Sonopuls, Germany). The encapsulation efficiency of the formulation for the probiotic bacteria was determined according to the formula: Encapsulation efficiency (%) = Bacteria in the capsules (CFU/ml) / Bacteria in initial cell suspension (CFU/ml) × 100.

"Evitalia" probiotic cells (PC) were grown on three different nutrient media: MRS medium, milk medium, and glucose-peptone medium. The largest number of LAB cells was formed when growing on MRS medium after 3 days of cultivation. In experiments on encapsulating bacterial cells, we used 3-day-old cultures of "Evitalia." The morphological and structural-mechanical characteristics of PGPs and particles loaded with the "Evitalia" LAB cells were studied. The degree of encapsulation of the probiotic LAB in PGPs was studied. More effectively, the LAB cells are encapsulated in PGPs formed from CP. The degree of loading of wet pectic particles from CP was 17.5%, and less for dry pectic particles based on CP (1.96%). Similar indicators for PGPs formed on the basis of AP were 5.6% and 0.33%, respectively.

Conclusion: PGPs formed on the basis of AP and CP can serve as a matrix for encapsulating probiotic LAB. (**International Journal of Biomedicine. 2022;12(3):450-453.**).

Keywords: probiotic "Evitalia" • encapsulation of lactic acid bacteria cells • apple pectin • citrus pectin • pectic gel particles

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Abbreviations

AP, apple pectin; **CP**, citrus pectin; **CFU**, colony-forming units; **LAB**, lactic acid bacteria; **PGPs**, pectic gel particles; **PC**, probiotic cells.

Introduction

The human gastrointestinal tract contains hundreds to thousands of different bacterial species that form a complex ecosystem of microorganisms that co-exist with the human host.[1,2]

Currently, more than 70 oral probiotics are produced worldwide.[3] A probiotic "Evitalia" produced in Russia (NPF PROBIOTIKA) is a complex of freeze-dried microorganisms that have retained the ability to multiply in the digestive tract of special strains of lactic acid microorganisms and producers of vitamins (B_1, B_2, B_6, B_{12}, A, E, C), folic acid, trace elements

of iron, calcium, and magnesium. The main feature of this association of microorganisms is their ability to ferment carbohydrates without forming gas, but with the formation of acids that acidify the contents of the intestine, inhibiting the growth of putrefactive and conditionally pathogenic microbes. Lactic acid bacteria (LAB) reduce the load on the liver by reducing the formation of amines, enterotoxins and other substances of microbial origin, which has a beneficial effect on increasing overall human resistance.

Over the past decades, there has been a tendency to produce functional food products containing probiotic bacteria. Probiotic bacteria are used in the production of functional foods and pharmaceuticals.[4,5]

Probiotics are defined as live microorganisms that, when administered in adequate amounts, provide health benefits to the host, including inhibiting pathogen growth, maintaining healthy intestinal microflora, and stimulating the immune system.[6,7]

For probiotics to have a positive effect on health, viable cells must have at least 7 log CFU after passing through the gastrointestinal tract before entering the colon. However, probiotics are vulnerable to stress and harsh conditions in the gastrointestinal tract (extreme pH values, bile acids, etc.). The strategy to protect probiotics during transit through the gastrointestinal tract is to encapsulate them.[8]

It should be noted that any probiotic delivery system should be designed so that probiotics are released in the colon and can have a beneficial effect. Moreover, probiotics must be able to adhere to the colon mucosa and colonize it; otherwise, they will quickly pass through the human body. The viability of probiotics can be improved by embedding them in microgels or other types of microcapsules.[9]

Encapsulation techniques for protecting probiotic bacterial cells have led to a significant increase in the viability of these microorganisms in food, as well as in the gastrointestinal tract.[10] Most encapsulation technologies rely on the immobilization of probiotic bacteria in a polymer matrix that retains its structure in the stomach until it is degraded and dissolved in the intestine.[11]

The purpose of this work was to research the encapsulation of the "Evitalia" LAB in pectic gel particles (PGPs) formed on the basis of apple pectin and citrus pectin.

Materials and Methods

Commercial apple pectin (AP) AU701 (Herbstreith & Fox KG, Germany) and citrus pectin (CP) CU701 (Herbstreith & Fox KG, Germany) were used. For encapsulation in PGPs, a complex of "Evitalia" dry probiotic microorganisms was used, which consists of freeze-dried strains *of Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus helveticus,* and *Propionibacterium freudenreichii ssp. shermanii*. One bottle of "Evitalia" contains more than 4 billion live microorganisms.

"Evitalia" probiotic cells (PC) were grown on three different nutrient media: De Man's, Rogoza's and Sharp**'s** (MRS) medium,[12] milk medium, and glucose-peptone medium.[13] We used a modified MRS medium of the following composition (g/l): yeast extract – 4.0; casein hydrolysate – 10.0; glucose – 20.0; di-substituted ammonium citrate – 2.0; sodium acetate – 5.0; tween 80 – 1.0; K_2HPO4 – 2.0; $MgSO_4$ x $7H_2O$ – 0.2; $MnSO_4$ x $4H_2O$ – 0.05. In the medium, meat broth (100 ml/l) was used instead of meat extract (10.0 g/l). Meat broth was prepared as follows: to 500g of low-fat minced meat, 1.0L of water was added, incubated for 2 hours at a temperature of 37-39°C, filtered through a sieve, then the filtrate was boiled for 30 minutes, filtered through cheesecloth and the volume was adjusted to 1.0L. pH was adjusted to 6.8-7.0 and autoclaved for 15 minutes at a temperature of 121°C. The milk medium had the following composition (g/l): skimmed powdered milk – 100.0; sodium citric acid – 1.5; glucose – 10.0. Glucose-peptone medium had the following composition (g/l): peptone – 5.0; glucose – 10.0; NaCl – 5.0. To obtain dense media, 30 g/l of agar was added to the liquid media.

The CFU number in suspensions of PC was determined using the standard method of 10-fold dilutions and subsequent inoculation on agarized media: MRS, milk medium, glucose-peptone medium.

In the study of probiotic-encapsulated PGPs, they were preliminarily destroyed by ultrasound in an HD2070 ultrasonic homogenizer (Sonopuls, Germany). For these purposes, the probe of an ultrasonic homogenizer was placed in a 10mL test sample, which was irradiated for 15 min. The duration of exposure to ultrasound and its power were selected empirically, achieving the destruction of gel particles and maintaining cell viability.

Gel particles were prepared from 3% aqueous solutions of pectins in the presence of 0.34M $CaCl_2$ by ionotropic gelation.[14-17] The pectin samples were dissolved in distilled water and slowly stirred on a magnetic stirrer MM-5 at room temperature until completely dissolved. Spherical PGPs were prepared as described in our previous articles.[15-17]

An extrusion method was used to encapsulate PC in PGPs. In the initial suspensions of PC (6.92×10^8 CFU/ml for AP-based gel particles and 9.71×10^8 CFU/ml for CP-based gel particles), 60 mg of the corresponding pectin was added in a volume of 2 ml, respectively, and dissolved by slow stirring on a MM-5 magnetic stirrer (Russia) at room temperature until completely dissolved. The resulting mixture of probiotic and pectin was extruded using a syringe in the form of individual droplets through a needle with a hole diameter of 0.7 mm at a distance of 4-5 cm into a slowly stirred calcium chloride solution and further stirred for 20 minutes at room temperature. Then the formed gel particles loaded with PC were washed three times in distilled water with stirring for 5 minutes and dried for 10-14 hours at 37°C.

The diameter and density of PGPs were determined using an optical microscope (ALTAMI, Russia) with a camera and an image analysis program (ImageJ 1.46r program, National Institutes of Health, USA). For calibration, a linear scale was used; one pixel corresponded to 0.024 mm.

The encapsulation efficiency of the formulation for the probiotic bacteria was determined according to the formula: Encapsulation efficiency (%) = Bacteria in the capsules (CFU/ml) / Bacteria in initial cell suspension (CFU/ml) × 100.[18]

The statistical analysis was performed using the statistical software BioStat (version 4.03) and Microsoft Office Excel 2007.

Results and Discussion

In recent years, research has been carried out to test the possibility of using pectin hydrogels as carriers for a probiotic delivery system to the colon.[8,19]

At the first stage of our work, we studied the growth of probiotic LAB on various nutrient media. Table 1 shows the number of CFU during the growth of the "Evitalia" LAB on three different nutrient media: MRS medium, milk medium, and glucose-peptone medium.

Table 1.

The number of CFU during the growth of the "Evitalia" LAB on three different nutrient media.

Nutrient medium	CFU/ml
MRS	$2.9\pm0.2 \times 10^5$
Milk medium	$8.8\pm0.3 \times 10^5$
Glucose-peptone medium	$1.7\pm0.2 \times 10^5$

Approximately the same number of probiotic bacterial cells accumulate on all three nutrient media. However, to obtain suspensions of "Evitalia" cells, we chose the MRS medium, since when cultivating the probiotic on it, the most homogeneous culture broths are obtained.

The dynamics of the PC accumulation during its growth in a liquid MRS medium was studied. Figure 1 shows the growth of the "Evitalia" LAB on MRS medium during 5 days of incubation.

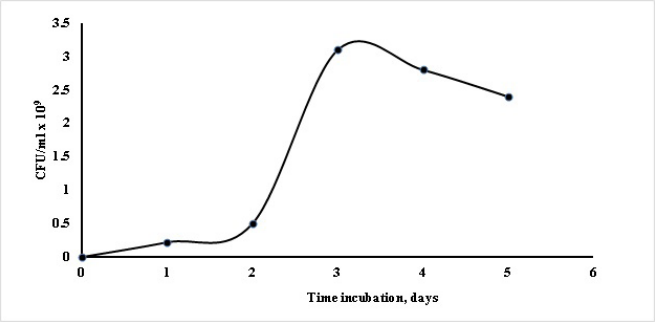

Fig.1. *Growth of the "Evitalia" LAB on MRS medium.*

The largest number of LAB cells was formed when growing on MRS medium after 3 days of cultivation. In experiments on encapsulating bacterial cells, we used 3-day-old cultures of "Evitalia."

Morphological and structural-mechanical characteristics of PGPs were studied. Table 2 shows the morphological and structural-mechanical characteristics of PGPs and particles loaded with the "Evitalia" LAB cells.

The diameter of the probiotic-loaded wet gel particles formed on the basis of CP (3.83 mm) was larger than the diameter of the AP gel particles (3.18 mm) loaded with LAB cells. For dry gel particles loaded with LAB cells, the particle diameter was larger for particles formed on the basis of AP (1.16 mm) than on the basis of CP (1.09 mm). The highest density (0.76 mg/mm^3) was found in probiotic-loaded dry gel particles formed from CP.

Table 2.

Morphological and structural-mechanical characteristics of PGPs and particles loaded with the "Evitalia" LAB cells.

Gel particles	Wet particles		Dry particles	
	Diameter, mm	Density, mg/mm^3	Diameter, mm	Density, mg/mm^3
AP	4.28±0.25	0.45±0.05	1.23±0.05	0.37±0.07
AP – «Evitalia»	3.18±0.15	0.59±0.10	1.16±0.14	0.60±0.05
CP	4.03±0.20	0.42±0.08	1.19±0.05	0.46±0.08
CP – «Evitalia»	3.83±0.10	0.58±0.09	1.09±0.06	0.76±0.09

The degree of encapsulation of the probiotic LAB in PGPs was studied. Table 3 shows the degree of loading of PGPs with "Evitalia." More effectively, the LAB cells are encapsulated in PGPs formed from CP. The degree of loading of wet pectic particles from CP was 17.5%, and less for dry pectic particles based on CP (1.96%). Similar indicators for PGPs formed on the basis of AP were 5.6% and 0.33%, respectively.

Table 3.

The degree of loading of PGPs formed from 3% AP AU701 and from 3% CP CU701, "Evitalia" probiotic cells.

Gel particles	The original cell suspension, CFU/ml	Gel particles with encapsulated PC, CFU/ml	PGP loading rate with "Evitalia," %
AP wet	6.92×10^8	3.9×10^7	5.60
AP dry	6.92×10^8	2.3×10^6	0.33
CP wet	9.71×10^8	1.7×10^8	17.5
CP dry	$9.71 \times x\ 10^8$	1.9×10^7	1.96

Thus, our research shows that pectic gel particles formed on the basis of apple pectin and citrus pectin can serve as a matrix for encapsulating probiotic lactic acid bacteria.

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***Corresponding author:** Elena A. Mikhailova. Institute of Physiology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS. Syktyvkar, the Russian Federation. E-mail: elkina@physiol.komisc.ru*

Competing Interest

The authors declare that they have no competing interests.

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